EXCYSTMENT IN THE CILIATE BURSARIA TRUNCATELLA

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Introduction

The resting cyst of *Bursaria truncatella* was first described and figured by Cienkowsky (1855). Further observations were made by Brauer (1886), Bütschli (1887–89), Lund (1917), Penard (1922), Kahl (1932), and Poljansky (1934), so that its general structure is well-known. The cyst is spherical and has only two clearly recognizable membranes. Perhaps a third is present, since Poljansky, by treating the cysts with hypertonic salt solutions (method of Ilowaisky, 1926), was able to observe on the surface of the shrunken protoplast delicate folds which he interpreted as an innermost membrane or intimocyst. Whether these folds represent a valid membrane or a surface differentiation of the cytoplasm is not clear. The two readily observable membranes are a thin, inner endocyst and a thick, outer ectocyst whose relation to the inner membrane confers on the cyst its peculiar features of structure. The outer surface of the ectocyst is not smooth, but faceted. The center of each facet is depressed and is attached to the endocyst by a short, cylindrical bridge (Fig. 4). The bridges have been referred to by a variety of names, among them, Stäbchen (Brauer), Stränge and Faden (Bütschli), tubes (Penard), Balken (Kahl), and Füsschen (Poljansky). For brevity and accuracy Bütschli's description of the cyst (p. 1726) can scarcely be surpassed: "Cyste kuglig, mit doppelter Hülle; die innere an mehreren Punkten an der äusseren befestigt und letztere daher an diesen Stellen dellenartig eingezogen." Thus the ectocyst presents externally many roughly circular or polygonal depressions which are separated one from another by ridges. The ectocyst is apparently unattached to the endocyst in the areas between the bridges. It can be readily separated from the endocyst, according to Brauer, leaving the bridges attached to the latter. Thus the bridges appear to be derivatives of the endocyst.

One of the facets is somewhat larger than the others and bears what appears to be an unusually large bridge. Various names have been applied to this structure: Pfropf (Bütschli), hilum (Lund), collerette (Penard), and Cystendeckel (Poljansky). The use of these varied terms indicates that its true nature has not been clearly recognized. Its significance becomes evident only when the process of excystment is observed. Then it proves to be analogous to the micropyle of a Spongillid gemmule; it is an aperture of exit or emergence pore. Although the terms Pfropf and Cystendeckel suggest such a function, I have been unable to find in the literature a description of the actual excystment process. The present paper describes the process and includes some further observations on the properties and structure of the cysts.

Material; Method of Inducing Encystment

In late August 1946, *Bursaria truncatella* appeared in considerable numbers in two large culture dishes in the laboratory. These contained the water, bottom sediment, and usual detritus of a recent collection from a temporary pool near Chapel Hill, North Carolina. With the addition of a little hay infusion from time to time *Bursaria* flourished in the dishes for a month, feeding largely on *Arcella, Colpidium* and *Paramecium bursaria* and dividing to all appearances exclusively by night. This peculiar habit of nocturnal division was noted by Schmähl (1926) and is unexplained. Evidently it is not correlated with the character of the food, since Schmähl's specimens fed largely on *Urocentrum, Stentor* and *Frontonia*. Individuals taken from the culture dishes during the day measured 400–550 μ by 225–325 μ and were therefore of average size for the species. Attempts to subculture them in smaller dishes and depression slides were unsuccessful.

Cysts were obtained by depriving active specimens of food in spring water. Usually groups of 10 or 20 individuals were removed to 0.75-cc. amounts of spring water in Columbia culture dishes. Again, groups of 50 were removed to 2-cc. amounts in Boveri dishes. After 24 hours the dishes always contained cysts exclusively, in number equal to or slightly less than the original number of specimens. Experience showed that the bursarias were easily injured by manipulation in small pipettes. Injured specimens disintegrated readily, and thus accounted for the disappearance of certain individuals. However, the losses from mechanical injury were low; for example, 13 dishes into which 260 bursarias were removed on August 30 contained 254 cysts on the following day. In general, one active specimen could be depended on to produce one cyst, lightly attached to the bottom of the dish. Since none remained unencysted, the encystment rate must be regarded as 100 per cent. Encystment usually occurred between the 6th and 12th hours of starvation. On many occasions a single active specimen was isolated in 0.75 cc. of spring water. A specimen so isolated always produced one cyst. Hence crowding, of primary importance in inducing encystment in *Colpoda* and *Didinium* (Barker and Taylor, 1931; Beers, 1947), plays no significant role in the encystment of *Bursaria*. Some 5000 cysts were obtained in August and September by the method outlined above. By means of a blunt needle they were detached from their original dishes and were stored in spring water in a smaller number of containers, some in stoppered vials, others in watch glasses kept in moist chambers. These cysts constituted the material on which the excystment experiments were based. Their history will be resumed shortly. At this point a further word on the factors responsible for encystment, followed by brief mention of some structural features of the cysts, seems in order.

Relatively little is known regarding the factors that induce encystment in *Bursaria*. Poljansky merely remarks that unfavorable conditions lead to encystment. Schmähl noted that specimens encysted after a variable number of days if kept at 4–7° C. in the presence of ample food, or if kept in continuous darkness, likewise with ample food. Hence, he cited low temperatures and darkness as encystment-inducing factors. However, neither of these factors was operative in the present study. Active specimens removed to spring water at 10:00 A.M. and kept at 22–24° C. in the natural light of the laboratory were always beginning to encyst at 5:00 P.M. Specimens removed to spring water at 5:00 P.M. encysted during the

night. Changes in pH did not account for encystment, since the pH of the spring water (6.6) was practically the same as that of the culture fluid from which the bursarias were taken (6.4–6.8). Although the salt concentration of the spring water was undoubtedly well below that of the cultures, encystment could not be explained on the basis of a decrease in salt concentration for the following reason. When all the food organisms were removed centrifugally from a sample of fluid from one of the cultures, the bursarias encysted as readily in this fluid as in spring water. Hence, the evidence indicates that absence of food was the primary, and likely the sole factor responsible for encystment.

Some Observations on the Structure of the Cysts

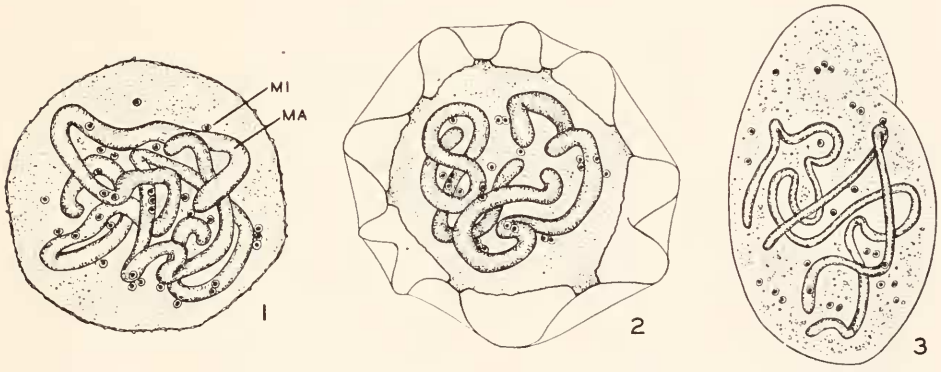
The diameter of the protoplast, as measured in the living condition, varied from 120 μ to 200 μ in the cysts at my disposal. Usually it was in the neighborhood of 155 μ when a sample of 20 or more cysts was measured. In Poljansky's cysts it averaged 167 μ. The thickness of the membranes was difficult to measure accurately because of their uneven external surface. It varied from 15 μ to 30 μ in different cysts; the mean was about 20 μ. Thus the membranes added about 40 μ to the diameter of the protoplast. The bridges usually measured 11–14 μ in diameter and 4–13 μ in length. As a rule they were short, but in some 4-month-old cysts they had a length of 15 μ and resembled short columns with broadened ends. Bridges of this type, but apparently even more elongated, are shown in Kahl's figure (p. 478). The number of bridges varied; some cysts had 40 or more. The diameter of the emergence pore varied from 20 μ to 30 μ. By reflected light the cysts appeared to be white. By transmitted light they looked black; actually they were opaque because of the immense number of granules in the cytoplasm. The granules were so plentiful that the macronucleus was quite obscured by them.

The emergence pore of the *Bursaria* cyst is closed by a thin, inelastic membrane (opercular membrane) which rests upon a low collar. Just beneath the emergence pore there is an area of hyaline, granule-free cytoplasm, though in my experience it is not always as conspicuous as the figures of Brauer and Lund indicate. Its significance is not understood, but since it is always prominent in the early stages of excystment (Fig. 5) it may be concerned with the elaboration of a substance which weakens the opercular membrane. It is evident that the emergence pore and underlying hyaline area give to the cyst an axis of polarity. Evidence deduced from the manner of emergence, to be presented later, indicates that the position of the emergence pore corresponds with the posterior end of the animal.

The nuclear structure and interrelations are well known for active bursarias, thanks to the work of Schuberg (1887), Schmähl, and Poljansky. The macronucleus has the form of a long, slender, variously contorted rod, whose total length often exceeds that of the animal. In some specimens it is in two parts. The micronuclei are small spheres, 4–5 μ in diameter and scattered indiscriminately in the cytoplasm. In stained preparations each appears as a central, deeply staining body surrounded by a clear area which is bounded externally by the nuclear membrane. Poljansky regards the clear area as a fixation artifact. The number of micronuclei is variable; Lund counted 9 to 15 or more; Schmähl, 20 or more; Poljansky, 15 to 34; my specimens had 16 to 28.

In order to examine the nuclear relations within the cysts, some of the active bursarias from the culture dishes were allowed to encyst on cover slips. These were immersed in Schaudinn's fluid (with acetic) at different stages of encystment and the specimens were stained by the Feulgen method. Individuals in the early stages of encystment remained attached and stained well. Individuals with fully developed cyst membranes usually became detached during the acid hydrolysis and had to be handled from this point in embryological watch glasses. The impervious nature of the mature cyst membranes introduced difficulties in staining, so that the final preparations of mature cysts were barely adequate to reveal the nuclei. Later, when it became possible to activate the cysts dependably, some of them were fixed and stained at various stages of emergence.

Whether a macronuclear reorganization occurs within the cysts was a point of special interest. If such a reorganization is present in ciliate cysts, it usually occurs in the early stages of encystment (Burt, Kidder and Claff, 1941; Beers, 1946). However, an examination of a considerable number of immature cysts (fixed 6–12 hours after isolation in spring water), as well as mature cysts (fixed 18, 24, or 48 hours after isolation), failed to reveal any evidence of a reorganization. Nevertheless, brief mention of the complex configuration of the macronuclear loops and of the number and spatial distribution of the micronuclei seems appropriate, since these features of the cyst have received little more than incidental mention in the literature.



FIGURES 1–3. *Bursaria truncatella.* Nuclear relations during encystment and excystment. From Schaudinn-Feulgen preparations. Camera lucida.

FIGURE 1. Young cyst. Macronucleus somewhat closely coiled; most of the micronuclei lying alongside macronucleus.

FIGURE 2. Immature cyst. All micronuclei now close to macronucleus. These relations are retained in the mature cyst.

FIGURE 3. Excysted specimen, immediately after emergence. Macronucleus in form of open loops; micronuclei scattered in cytoplasm. The macronucleus, whether in cysts or active specimens, may be in one part or in two.

In general the macronucleus retains throughout the encystment and excystment periods the type of structure already described for the trophic ciliate, although it assumes in the cyst the form of such close coils and loops that its parts may become actually intertwined (Figs. 1 and 2). Upon excystment the loops become more open, as formerly (Fig. 3). As to the micronuclei, they are scattered

through the cytoplasm in pre-cystic (unattached or lightly attached) specimens, quite as in trophic individuals. As encystment proceeds, they assume a position close to the macronucleus, though distributed seemingly at random along its length (Figs. 1 and 2). This position is retained until excystment occurs, when they scatter (Fig. 3). There is no evidence of micronuclear division during encystment, and the number of micronuclei within the cysts agrees with the number found in active specimens.

Attempts to Induce Excystment; Final Success

To return to a consideration of the cysts which were stored in spring water, many attempts were made between September 15, 1946, and June 1, 1947, to induce the emergence of samples of them. The procedure followed was similar to the one employed successfully in previous studies on the excystment of *Tillina* and *Didinium* (Beers, 1945, 1946a): 10 or 20 cysts were first washed in the medium to be tested and were then removed to 0.75 cc. of the medium in a Columbia culture dish. A detailed description of these experiments or a presentation of the results in tabular form would be little more than a wearisome chronicle of failure. The experiments will receive only brief consideration.

Samples of cysts stored at room temperature were tested when they attained the following ages: 1 week, 2 weeks; 1, 3, 5, 6, and 8 months. The following fluids were used: various concentrations of freshly prepared infusions of dried lettuce, some unbuffered, others buffered from pH 6.0 to 8.0 with phosphate buffer mixture, some at 22° C., others at 28° and 32° C.; similar concentrations of timothy-hay infusion, likewise varied with reference to pH and temperature; various concentrations of Difco yeast extract and peptone in aqueous solutions; the foregoing media pre-inoculated with wild bacteria; distilled water; spring water containing a dense population of *Paramecium caudatum*. Other samples were allowed to dry gradually on filter paper before being tested; still others were subjected to cold treatment (1 month at 15° C. followed by 3 months at 5° C.), in an effort to duplicate their natural winter surroundings. Of the dried and cold-treated cysts (about 500 of each were tested), none emerged. Of the cysts stored at room temperature (about 3000 were tested), the following 8 excysted. Three, age 1 month, emerged in unbuffered 0.1 per cent lettuce infusion at 22° C. and one of the same age emerged in similar infusion buffered at pH 8.0. One, age 3 months, excysted in 0.5 per cent lettuce infusion at 22° C. and 2, of similar age, in 0.2 per cent hay infusion at 28° C. Another, age 6 months, came out in 0.5 per cent lettuce infusion at 28° C. Excystment occurred between the 8th and 24th hours. It should be understood that a particular sample of cysts was tested in only one fluid and was then discarded, since an attempt was being made to discover a medium which had reliable excystment-inducing properties.

Even though the vast majority of the cysts failed to become active, I was not in the least disposed to regard them as dead. The dense granulation and the opacity of the cysts have been mentioned. The characteristic, uniformly opaque appearance was retained by practically all the cysts, with no indications of disintegrative changes. However, at least 6 of the 8 cysts that emerged, it was noted by chance, presented an exceptional appearance with reference to granulation and opacity. They had become less densely granular, light brown in color, and transparent to

the extent that the macronucleus was faintly visible. They were assumed to be abnormal, and it was fortunate that they were tested for excystment and not discarded, for their emergence furnished the first tangible clue to a solution of the immediate problem. It indicated that cysts of *Bursaria* undergo with the passage of time a gradual, intrinsic physiological change which renders them capable of excystment. With this possibility in mind, the remaining cysts, about 750 which had been stored at room temperature and 250 which had received cold-treatment, were examined in the early part of June, but practically none showed a decrease in opacity.

Shortly thereafter they were transported to the Mt. Desert Island Biological Laboratory, where they remained undisturbed until August 12. On this date, when they were about 11 months old, an examination showed that fully 80 per cent of both lots had become light brown and semitransparent in varying degrees. Samples of them, upon immersion in lettuce infusion (0.1 gm. dried lettuce boiled 5 minutes in 100 cc. distilled water, cooled and used at once), excysted regularly. Tests were made at 22° and 32° C. At either temperature practically 100 per cent of the cysts became active within 2–6 hours. Both lots responded equally well, so that the cold-treatment was without effect. Other samples tested in higher concentrations of lettuce infusion (up to 1 per cent) and in hay infusion (0.1–2.0 per cent) likewise excysted dependably.

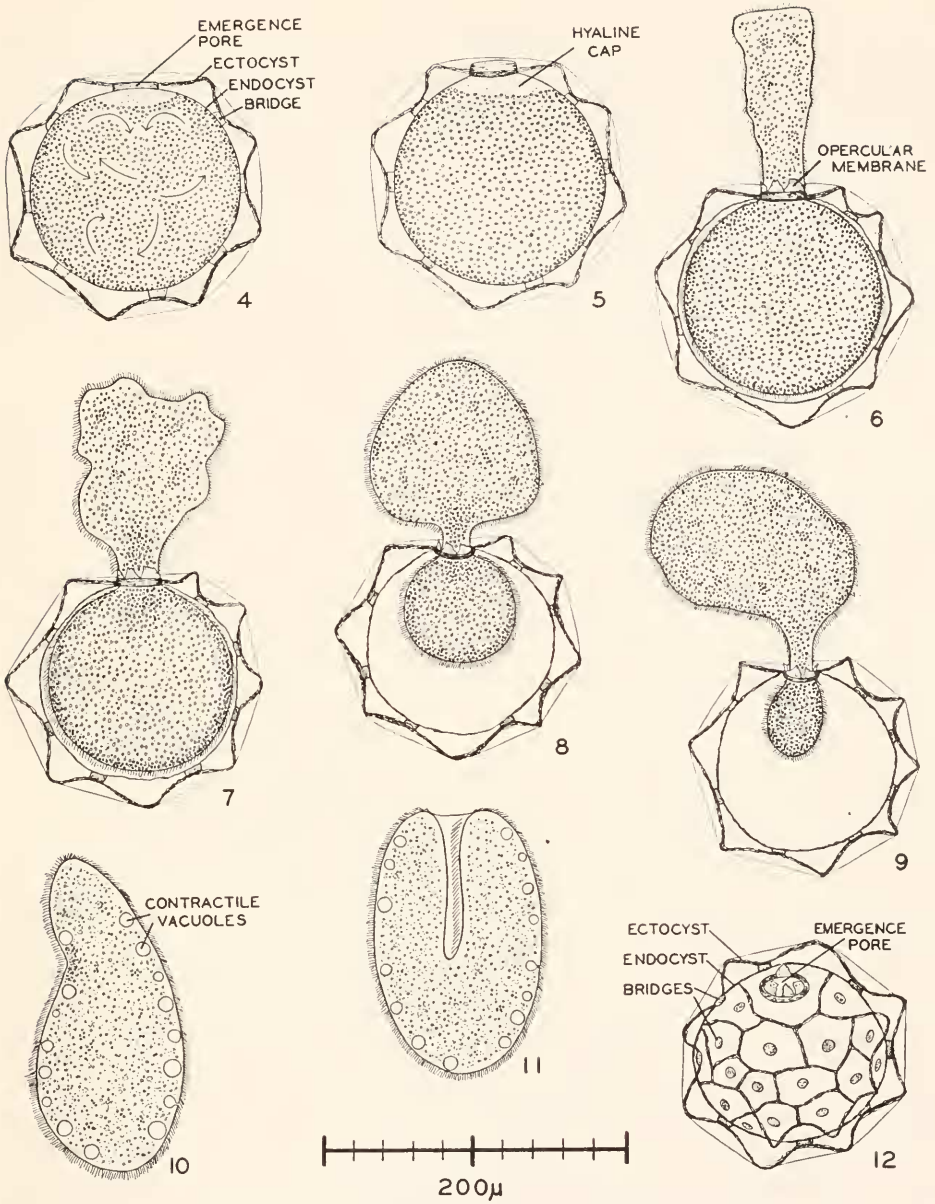
With favorable cysts and suitable excysting media at hand, it became possible to observe the entire excystment process as often as desired. After an immersion of 2 hours in lettuce infusion in an open dish, the cysts were mounted in infusion under a supported cover slip and the sequence of events was recorded with the aid of a camera lucida.

The Excystment Process

The excystment of *Bursaria* borders on the incredible. It is fully as amazing as the ingestion of *Paramecium* by *Didinium* or of *Closterium* by *Frontonia*. The details of the process follow, though it is understood that the time intervals cited may vary in different cysts.

Cyclosis begins after an hour in lettuce infusion and becomes more vigorous within the next hour. The cytoplasmic streams follow no regular path (Fig. 4). At the end of 2 hours the hyaline area under the emergence pore is conspicuously developed and resembles a transparent cap resting on the subjacent granular cytoplasm. Its base is always clearly delimited from the underlying granules (Fig. 4). The granules may oscillate near or stream by its base, but they do not enter it. It seems to be a region of gelated, granule-free cytoplasm. The opercular membrane now begins to bulge outward, and the collar of the emergence pore appears to be taller (Fig. 5). The cyst, as measured along its axis of polarity, has increased slightly in length at this stage. It is evident that considerable pressure is being exerted from within and that the emergence pore is the weakest area in the cyst wall. Suddenly the opercular membrane gives way and a column of cytoplasm protrudes eruptively (Fig. 6). In general the column is cylindrical, though its free end is sometimes broadened momentarily. The hyaline cap is lost sight of as the cytoplasm erupts, and the distal end of the column becomes highly ameboid. Indeed, the cytoplasmic column erupts so suddenly and changes shape so rapidly

that this phase of the excystment process could be recorded faithfully only by cinephotomicrography, not by pencil and camera lucida. With the release of internal pressure the cyst membranes resume the shape of a sphere and a space appears between the endocyst and protoplast. The remains of the opercular membrane project outward as short jagged flaps from the collar of the emergence pore. Cytoplasm and granules now begin to stream through the emergence pore much as the plasmasol flows forward in an actively moving monopodal ameba. Thus the original column increases rapidly in volume, becoming bulbous and expanded in the meantime (Fig. 7). It changes shape constantly, rapidly extending and retracting numbers of lobose, pseudopod-like processes, as if in a state of great morphological instability, though the portion enclosed by the membranes remains spherical and relatively passive throughout the entire process of emergence. The presence of cilia can now be detected for the first time on the organism, and the cilia aid it in its further progress outward. By means of its cilia it retreats a short distance into its membranes and then delivers a vigorous thrust outward against the emergence pore. Each such outward thrust or lunge forces a little more of its surface area through the emergence pore, while its inner substance streams uninterruptedly through the pore. Thus two mechanisms are now operative in its emergence: ciliary action, which forces its cortical substance through the emergence pore by initiating vigorous outward thrusts; and endoplasmic streaming, which transports outward much of its inner substance, including the macronucleus. When the organism is approximately half excysted, it has roughly the shape of a dumbbell (Fig. 8). As the condition of final liberation nears, the free portion becomes unusually active. It changes shape constantly. At times it elongates, again it flattens on the slender, constricted, stalk-like portion and assumes the shape of the cap of a mushroom. It bends, first in one direction, then in another (Fig. 9), and twists through as much as 180 degrees on the stalk. Thus it becomes incredibly distorted, but as the last portion slips through the emergence pore these contortions end and it becomes pyriform in shape (Fig. 10). The first portion to emerge constitutes the rounded body, the last portion the neck of the pyriform ciliate. After a brief pause, the ciliate swims away, always with the pointed end, which emerged last, directed forward. Thus the posterior end lies immediately beneath the emergence pore and is the first part to emerge, whereas the anterior end emerges last. The contractile vacuoles show with great clarity now, and they are functional. Forty to 80 may be counted with ease, and there are probably many more. There is no recognizable indication of a ventral furrow or peristome at this stage, although some of these structures are no doubt represented in a specialized area of cytoplasm which occupies a sort of notch at the base of the neck. At this stage (Fig. 10) the ciliate could never be recognized as a member of the genus *Bursaria*. During the next hour, while the ciliate spirals counterclockwise through the medium, with brief periods of rest, its development is completed. Its pointed anterior end broadens, its body becomes flattened, and the ventral furrow and peristomial membranelles develop (Fig. 11). When these changes have been completed, the ciliate is ready to begin feeding. If food is not supplied, the excysted specimens re-encyst, forming smaller, light brown, semi-transparent cysts whose protoplasts measure only 95–120 μ in diameter. Following emergence the old cyst membranes are left intact in the medium (Fig. 12), and the number of bridges can be counted with relative ease.



FIGURES 4–12. Excystment in *Bursaria truncatella*. The figures show successive stages in the emergence of the same individual; drawn from life with the aid of the camera lucida.

FIGURE 4. Cyst after 2 hours in excystment fluid. Arrows indicate direction of cyclosis.

FIGURE 5. Organism almost ready to break through emergence pore.

FIGURES 6–9. Escape by way of emergence pore.

FIGURE 10. The ciliate just after emergence—pyriform in shape and lacking ventral groove and membranelles.

FIGURE 11. Ventral groove and membranelles developing.

FIGURE 12. The empty cyst membranes.

The entire process of excystment and the subsequent differentiation require a minimum of 3 hours, counting from the time of immersion in the excystment medium. As has been said, 2 hours in the medium are usually necessary before the opercular membrane ruptures. Actual escape from the membranes requires only a few minutes. Thus the events shown in Figures 6–10 (rupture of the opercular membrane to final emergence) consume as a rule only 4 or 5 minutes. Subsequent differentiation requires 45 minutes to an hour. The foregoing time intervals are subject to much variation. Some cysts may remain 5 hours in the excystment medium before the opercular membrane tears. Some individuals require as much as 12 or 15 minutes to effect their escape. In general, it may be said that the time required for excystment at 22° C. varies from 2 to 6 hours.

Further History of the Cysts; Re-encystment and Re-excystment

On September 1, 1947, there remained in the vials about 200 opaque cysts which presumably had not yet attained the physiological condition necessary for excystment. These were taken back to Chapel Hill and were set aside until October 18. When they were examined on this date, all were light-brown in color and semi-transparent. They were divided into 10 groups of approximately 20 cysts each. On October 20 and 22 five groups were tested for excystment in each of the following fluids: 0.2 per cent aqueous peptone solution; 0.1 per cent aqueous yeast-extract solution (Difco); 0.2 per cent lettuce infusion, freshly prepared; 0.3 per cent timothy-hay infusion, freshly prepared; Pyrex-distilled water. Some of the results were somewhat unexpected. Not only did all the specimens excyst in the plant infusions and in yeast extract, as expected, but all of those in peptone solution and distilled water also excysted. The time required for excystment varied as usual from 2 to 6 hours. Because of the relatively small number of cysts available for use in these experiments, it was impracticable to determine statistically which of the five fluids was most effective, i.e., which induced the highest percentage of excystment in the shortest time. Inspection of the results indicates that the peptone solution was least effective; none of the specimens in peptone solution was active at the end of 2 hours, whereas 20 per cent or more were active in the remaining fluids at the end of this time.

Many of the individuals which excysted in distilled water burst soon after emergence and all swam sluggishly. They were discarded. Most of the remaining specimens were transferred from their respective excystment fluids to a mixture consisting of 9 parts of spring water and 1 part of 0.1 per cent lettuce infusion, in which they encysted in the next 24 hours. This mixture was selected because its pH and tonicity are generally favorable for ciliates, and it does not support intense bacterial growth, which is often deleterious to ciliates. There resulted about 100 small, light brown, semi-transparent cysts. When these cysts were one week old, they were tested for excystment in the five fluids mentioned in the preceding paragraph. Practically all of them excysted after 2 to 6 hours in these respective fluids. Thus they did not require a long period of rest in order to become excystable.

Discussion

Reference to the work of Poljansky shows that there are two major categories of resting cysts in *Bursaria truncatella*. These are the so-called neutral cysts and the exconjugant cysts, the latter being formed by exconjugants when food is lacking. The two types are essentially alike as regards shape, cytoplasmic structure, and character of the membranes. They differ in the structure of the macronucleus. The exconjugant cyst contains 4 spherical or ovoid macronuclear anlagen; they are derived from 4 of the 8 nuclei that result from the first 3 divisions of the synkaryon. Neutral cysts are produced by vegetative individuals and therefore contain the usual elongated, bent macronucleus. Poljansky's exconjugant cysts were slightly smaller than his neutral cysts, probably because the exconjugants had had no opportunity to feed. Excystment was not studied by Poljansky.

I have had no opportunity to observe exconjugant cysts. All the cysts of the present study qualify as neutral cysts, a designation which is retained chiefly for descriptive purposes. The present results show that not all neutral cysts are alike in their capacity to excyst. Although the results are inadequate to permit conclusive generalizations, they show that there are two types of neutral cysts. Following the precedent of Johnson and Evans (1940) in their study of the cysts of *Woodruffia metabolica*, I shall designate them as stable and unstable. Probably this practice is warranted only for convenience of description; parallel comparisons of the cysts of the two genera are scarcely justified, for the cysts are very different structurally and somewhat different physiologically; their similarity resides chiefly in the fact that the unstable cysts become active more readily than the stable ones. Thus the *Bursaria* cysts which were obtained in late summer and early autumn of 1946 qualify as stable cysts. Stable cysts contain plentiful food reserves, to judge by their granulation. Presumably they are produced in autumn in nature when food becomes scarce, following a long period of vegetative reproduction in summer, though pure-line studies are needed to establish this thesis. They must pass through a long period of dormancy, in excess of 9 months according to the present observations, before many of them become excystable. During this period they undergo a physiological change which expresses itself visibly in reduced granulation and loss of opacity. Once a state of excystability is reached in consequence of these intrinsic changes, they excyst readily when a change in the chemical or physical nature of their environment occurs. Perhaps such cysts should be called winter cysts.

If the individuals which emerge from stable cysts are induced to re-encyst immediately by lack of food, they form unstable cysts. These contain fewer cytoplasmic granules and presumably meager food reserves. A long period of dormancy is not required to render them excystable; they can emerge within a week (perhaps sooner) if the environment is changed.

At this point in the discussion of unstable cysts, the results of some preliminary, though nevertheless significant, experiments on *Bursaria* cysts need to be recounted. In early May, 1946, there appeared in collections from the pool mentioned earlier a moderate number of specimens of *Bursaria truncatella*. Some hay infusion was added and the bursarias survived in the dishes for 3 weeks, though they never became numerous. Some of the fluid from the dishes was passed

through Whatman No. 43 filter paper to remove the food organisms, and some 40 bursarias were transferred to this fluid. They encysted within 24 hours, producing cysts of average size. To judge by my sketches, the cysts were light in color and semi-transparent; at least the sketches show the macronucleus and indicate relatively sparse granulation. The existence of two types of neutral cysts was unrecognized at the time. When these cysts were 6 days old they were tested for excystment. Of 20 cysts treated with distilled water, 18 excysted; of 18 treated with 0.05 per cent lettuce infusion, 17 excysted. The precise time required for excystment was not recorded, but it was less than 8 hours. Thus the cysts, although not derived from immediately excysted specimens but from active, dividing specimens in mid-spring, qualified as unstable cysts. In all probability they were derived from recently excysted specimens which had passed through relatively few generations since emergence from stable cysts. Perhaps the unstable cysts should be called vernal cysts.

To summarize, the evidence indicates that *Bursaria* naturally produces stable cysts in autumn after a long period of vegetative reproduction, and unstable cysts in spring or at other times when relatively few generations have passed since emergence. Further investigations, including intensive culture work, are needed to clarify these points. Too little is known about exconjugant cysts to permit comment on their excystment.

To return to a consideration of the factors that induced excystment in this study, the emergence of *Bursaria* in fluids as dissimilar as peptone solution, lettuce infusion, and distilled water is not easily explained. Since no doubt exists concerning the hypotonicity of distilled water, excystment in this fluid may be readily explained in osmotic terms: water enters the organism, probably by way of the opercular membrane, and thus initiates its emergence. In all probability the remaining four fluids were also hypotonic. If it be assumed that excystment in these fluids was likewise initiated solely by osmotic phenomena, i.e., by hypotonicity, it is implied that the spring water in which the cysts were stored was not hypotonic. This implication seems ill-founded, in view of the low salt content of spring water. To explain the facts it seems necessary to assume that the *Bursaria* cyst reaches a state of equilibrium with the environment at the time of encystment. When this state of equilibrium is disturbed by the entrance of any of a number of kinds of ions or molecules into the cytoplasm, the organism responds by excystment. On the other hand, it is possible that these ions or molecules, instead of having a specific effect on the cytoplasm, merely alter the permeability of the plasma membrane or opercular membrane or both, and thus initiate excystment by allowing water to enter the cytoplasm. From this point of view excystment in any fluid, unless demonstrably hypertonic, could be interpreted in terms of osmotic phenomena.

Since ciliate cysts in general are never wholly separated from their immediate environment by impermeable membranes, it is more or less self-evident that they must be in some sort of physiological equilibrium with the environment; otherwise they would not remain in the encysted state. Furthermore, they have the capacity to reach this state of equilibrium in different environments; otherwise their encystment would be too restricted to have survival value. This is a manifestation of the adaptive property of protoplasm. For example, *Didinium* can form viable, normal cysts in 2 per cent hay infusion, in natural spring water, or in purely in-

organic "artificial spring water" (Beers, 1947). The encystment of *Bursaria* either in spring water or in the rich, organic infusion of a wild mass culture has been mentioned. Probably all ciliates have the ability to encyst under many diverse conditions.

As to the conditions of excystment, were cysts so highly specialized as to be capable of emergence solely under a restricted and highly specialized set of conditions, the survival of the species would be threatened, in that these conditions might rarely be encountered. Hence, cysts are able to become active under a variety of conditions, some unfavorable for growth, multiplication and survival, others favorable. Paradoxical though it may seem, it is a fact that an effective excystment-inducing medium is not always favorable for continued life. Thus *Didinium* excysts readily in peptone media in which the bacterial activity is so intense that the didinia are killed upon emergence (Beers, 1946a). *Tillina magna, Bursaria* and other ciliates excyst in distilled water, which contains no food and is so hypotonic that some of the specimens always burst upon emergence. On the other hand, these ciliates also excyst under favorable conditions—for example, *Didinium* in infusions containing paramecia as well as bacteria which serve as food for the paramecia, and *Tillina* in plant infusions which support the growth of its bacterial food-organisms (Beers, 1945). By excysting under a variety of conditions, some individuals perish, but others survive. It is doubtful that the excystment of free-living ciliates can always be explained in terms of the action of a few specific, excystment-inducing substances.

Summary

A supply of cysts of *Bursaria truncatella* was obtained in autumn by depriving active specimens of food in spring water. The cysts were densely granular and opaque. In the following months many attempts were made to induce the excystment of samples of them, but with practically no success. Examination of the remaining cysts when they were 11 months old showed that fully 80 per cent of them had become relatively sparsely granular and semi-transparent. They could be activated dependably in plant infusions which had been ineffective earlier. Two months later the remaining 20 per cent of the cysts became excystable, not only in plant infusions, but also in peptone solution, yeast-extract solution, and distilled water. The results indicate that in autumn after the passage of many generations *Bursaria* produces cysts which require a long period of dormancy before they become excystable. These may be called stable cysts, but not all cysts are of this type.

Specimens which emerged from stable cysts, if not fed, re-encysted. These cysts, as well as others which were produced by specimens collected in spring, did not require a period of dormancy, but could be excysted within a week or less. These may be called unstable cysts.

The excystment process is the same in either type of cyst. It involves the rupture of the membrane which covers a special emergence pore, and the escape of the organism by way of this pore. Excystment is a remarkable feat, in that the spherical, encysted bursaria, measuring about 155 μ in diameter, makes its way outward through a circular opening measuring only 25 μ in diameter. Upon emer-

gence, the organism is so different in shape and structure from a typical specimen that it could never be recognized as a member of the genus *Bursaria*. Its development is completed within an hour.

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